



VITELLOGENIN GENE EXPRESSION IN FATHEAD MINNOWS EXPOSED TO EE2 IN A WHOLE LAKE DOSING EXPERIMENT

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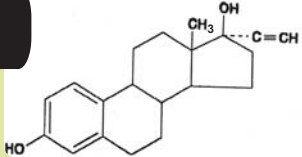
ABSTRACT

Vitellogenin Gene Expression in Fathead Minnows Exposed to EE2 in a Whole Lake Dosing Experiment.

A whole-lake endocrine disruption experiment was conducted by Fisheries and Oceans Canada at the Experimental Lakes Area (ELA), northwestern Ontario in 2001 and 2002. This experiment examined population, organism, biochemical and cellular-level effects in lake trout, white sucker, fathead minnow, and pearl dace exposed to environmentally-relevant, (4-6 ng/L) concentrations of the synthetic estrogen, 17 α -Ethinylestradiol. The USEPA collaborated in this study by evaluating vitellogenin gene expression (Vtg) in male fathead minnow livers: 1) from indigenous fish collected from the dosed and control lakes 4 times, once before dosing and up to three times after dosing; 2) In 2001 from indigenous minnows collected from the control lake and deployed for 1, 3, 7 and 13 days in the dosed and control lakes; and 3) In 2001 and 2002 from Cincinnati cultured minnows exposed to water collected from both ELA lakes and shipped to Cincinnati. RT-PCR methods were used for measuring Vtg expression. In addition to water exposures, embryo-larval fish were exposed to control and dosed lake sediment elutriates for 5 days. Gene expression in indigenous male fatheads collected at all time intervals from the dosed lake showed a constant level of elevation. Gene expression in the 2001 deployment study was detected within 24-hrs after deployment of control fish into the treated lake and stayed elevated for the entire 13-day study. Highly variable gene expression was found in fry exposed to dosed lake sediments but no significant gene expression was found in fry exposed to control lake sediments. Results indicate that RT-PCR analyses of total RNA can be used to provide a rapid and timely estimate of exposure to estrogenic substances.

This abstract has been Agency reviewed and approved for publication.

Why look at 17 α -ethinylestradiol (EE2)?



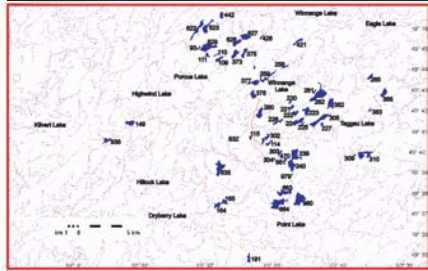
- effective component of birth control pills
- potent estrogen mimic
- 70-80 % degraded in sewage treatment found at significant and effective concentrations downstream of municipal wastewater treatment plants

Canadian DFO Fathead Endpoints

- Vitellogenin (spring, mid-summer, fall)—V. Palace
- Liver, kidney and gonad development, GSI and LSI (spring & fall)—B. Evans
- Secondary sex characteristics (mid-summer)—P. Blanchfield
- Male reproductive behaviour (mid-summer)—P. Blanchfield
- Nest size and egg development (mid-summer)—P. Blanchfield
- Population size structure, growth, abundance (spring and fall)—K. Mills

58 Designated Research Lakes and their Watersheds Detailed Monitoring since 1969

Located in northwestern Ontario approximately 250 km east of Winnipeg and 50 km east-southeast of Kenora.

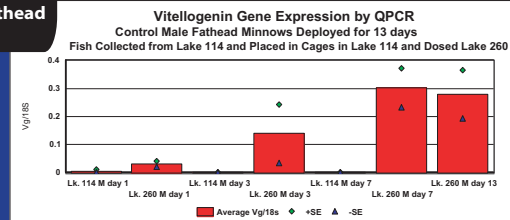


Boreal Shield of northwestern Ontario

EPA NERL 2001 Objectives

- Evaluate exposure of indigenous male fathead minnows in Lake 260 to ethinylestradiol using vitellogenin gene expression
- Evaluate short-term exposure of male fathead minnows from Lake 114 deployed in Lake 260
- Evaluate exposure of Cincinnati male fathead minnows to Lake 260 water at the US EPA facility in Cincinnati
- Evaluate exposure of Cincinnati fathead minnow fry to sediment from Lake 260

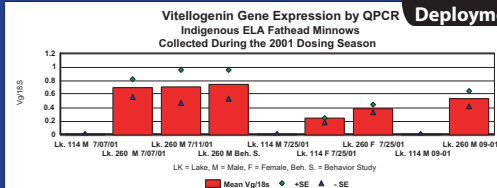
Figure 1
2001 Results of Indigenous Male Fathead Minnows



EPA NERL Approach - Deployment Study

- Fish were collected in Lake 114 using minnow traps two days before deployment.
- On day of deployment males were separated from females and placed in cages in Lakes 114 and 260.
- Minnows were retrieved from cages on days 1, 3, 7 and 13.
- Livers were collected and RT-PCR performed.

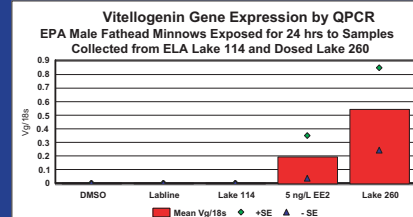
Figure 2
2001 Results of 13-Day Deployment Study



EPA NERL Approach - Grab Samples

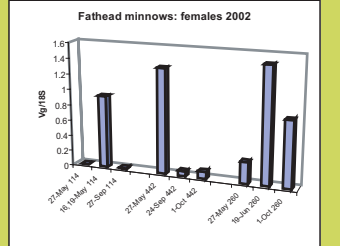
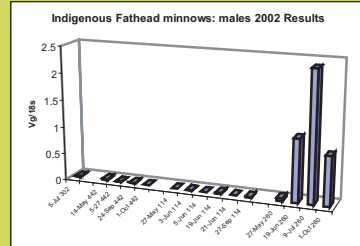
- Water was collected from Lakes 114 and 260. Samples were shipped to Cincinnati.
- Male Cincinnati fathead minnows were exposed to water samples for 48 hours with water renewal after 24 hours.
- Male fathead minnows were also exposed to 5 ng/L ethinylestradiol.
- RT-PCR was performed on RNA from liver samples.

Figure 3
2001 Comparison of Exposure of Minnows to Lakes 114 and 260 Grab Samples



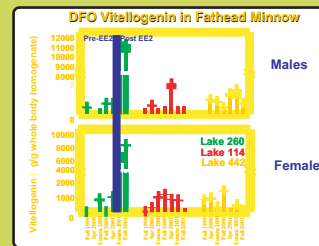
2002 Objectives Vitellogenin Expression Study

- Evaluate exposure of indigenous male fathead minnows in Lake 260 to ethinylestradiol using vitellogenin gene expression before and 3 times during the dosing.
- Evaluate exposure of Cincinnati male fathead minnows to Lake 260 water at the US EPA facility in Cincinnati
- Evaluate exposure of Cincinnati fathead minnow fry to sediment from Lake 260

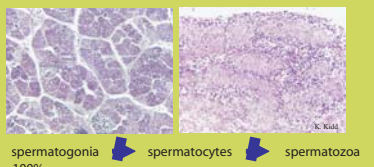


EPA Observations: 2002 fathead minnows

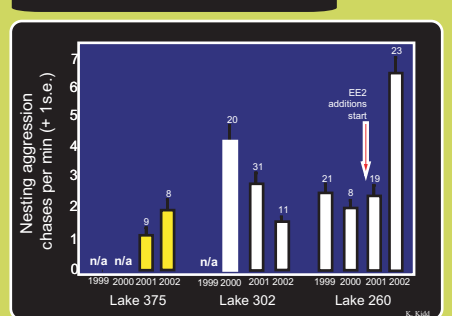
- Females in lake 260 continued to have higher vitellogenin gene expression into October, compared to Lake 114 and Lake 442 females
- A very impressive lack of vitellogenin gene expression in all males from all control lakes
- Males in lake 260 exhibited high vitellogenin gene expression after dosing, with expression remaining high at least until October 1



DFO Histopath Results Lake 260 Fathead Minnow Testes - Spring



DFO Male Fathead Minnow Behaviour Nest Defense Results



Summary DFO Results

- Spring 2001
 - EE2 additions began
 - significant vitellogenin induction after 7 weeks
- Fall 2001 (4 months)
 - proteinaceous accumulation in kidney
 - liver cell size increased
- Spring 2002 (12 months)
 - disorganized testes, immature ovaries
 - increased spawning aggression, fewer & less-developed eggs
 - reduced secondary sex characteristics
- Fall 2002 (17 months)
 - reproductive failure, few fish >1 year
 - Spring 2003 (2 years) –only age 2 fish remaining
 - one male found, females with large ovipositors
- Fall 2003 - complete collapse of population predicted

QPCR measurements and Analyses were performed by Ana Braam, Brandon Tonnis and Kristen Lipscomb
prepared by CSC Inc Graphics Support for U.S. EPA/NERL November 2003